



DEPARTMENT OF THE AIR FORCE

59TH MEDICAL WING (AETC)
LACKLAND AIR FORCE BASE TEXAS

18 MAR 2016

MEMORANDUM FOR SGVT

ATTN: LT COL MICHAEL R DAVIS

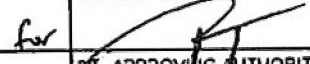

FROM: 59 MDW/SGVU

SUBJECT: Professional Presentation Approval

1. Your paper, entitled **Locally Applied Enzyme Activated Tacrolimus Eluting Hydrogels Significantly Delay the Onset of Acute Rejection of VCA Grafts** presented at **2016 Society of Military Surgeons, Boston, MA, 17-19 March 2016** with MDWI 41-108, and has been assigned local file #**16139**.
2. Pertinent biographic information (name of author(s), title, etc.) has been entered into our computer file. Please advise us (by phone or mail) that your presentation was given. At that time, we will need the date (month, day and year) along with the location of your presentation. It is important to update this information so that we can provide quality support for you, your department, and the Medical Center commander. This information is used to document the scholarly activities of our professional staff and students, which is an essential component of Wilford Hall Ambulatory Surgical Center (WHASC) internship and residency programs.
3. Please know that if you are a Graduate Health Sciences Education student and your department has told you they cannot fund your publication, the 59th Clinical Research Division may pay for your basic journal publishing charges (to include costs for tables and black and white photos). We cannot pay for reprints. If you are 59 MDW staff member, we can forward your request for funds to the designated wing POC.
4. Congratulations, and thank you for your efforts and time. Your contributions are vital to the medical mission. We look forward to assisting you in your future publication/presentation efforts.

Linda Steel-Goodwin

LINDA STEEL-GOODWIN, Col, USAF, BSC
Director, Clinical Investigations & Research Support

PROCESSING OF PROFESSIONAL MEDICAL RESEARCH/TECHNICAL PUBLICATIONS/PRESENTATIONS			
1. TO: CLINICAL RESEARCH	2. FROM: (Author's Name, Rank, Grade, Office Symbol) Michael R Davis, O-6, Lt Col, 59MDW ST	3. GME/GHSE STUDENT <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	4. PROTOCOL NUMBER. Navy 15-09
5. PROTOCOL TITLE: (NOTE: For each new release of medical research or technical information as a publication/presentation, a new 59 MDW Form 3039 must be submitted for review and approval.) Vascularized Composite Allotransplantation (VCA) in Swine (Sus scrofa) for Optimization of Reconstruction of Battlefield Injuries Using the			
6. TITLE OF MATERIAL TO BE PUBLISHED OR PRESENTED: Locally Applied enzyme activated tacrolimus eluting hydrogels significantly delay the onset of acute rejection of VCA grafts			
7. FUNDING RECEIVED FOR THIS STUDY? <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO FUNDING SOURCE: 59MDW ST			
8. DO YOU NEED FUNDING SUPPORT FOR PUBLICATION PURPOSES: <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO			
9. IS THIS MATERIAL CLASSIFIED? <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO			
10. IS THIS MATERIAL SUBJECT TO ANY LEGAL RESTRICTIONS FOR PUBLICATION OR PRESENTATION THROUGH A COLLABORATIVE RESEARCH AND DEVELOPMENT AGREEMENT (CRADA), MATERIAL TRANSFER AGREEMENT (MTA), INTELLECTUAL PROPERTY RIGHTS AGREEMENT ETC.? <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO NOTE: If the answer is YES then attach a copy of the Agreement to the Publications/Presentations Request Form.			
11. MATERIAL IS FOR: <input checked="" type="checkbox"/> DOMESTIC RELEASE <input type="checkbox"/> FOREIGN RELEASE CHECK APPROPRIATE BOX OR BOXES FOR APPROVAL WITH THIS REQUEST. ATTACH COPY OF MATERIAL TO BE PUBLISHED/PRESENTED.			
<input type="checkbox"/> 11a. PUBLICATION/JOURNAL (List intended publication/journal.)			
<input type="checkbox"/> 11b. PUBLISHED ABSTRACT (List intended journal.)			
<input type="checkbox"/> 11c. POSTER (To be demonstrated at meeting: name of meeting, city, state, and date of meeting.)			
<input checked="" type="checkbox"/> 11d. PLATFORM PRESENTATION (At civilian institutions: name of meeting, state, and date of meeting.) 2016 Society of Military Surgeons, Boston MA 11-16-Mar-2016			
<input type="checkbox"/> 11e. OTHER (Describe: name of meeting, city, state, and date of meeting.)			
12. EXPECTED DATE WHEN YOU WILL NEED THE CRD TO SUBMIT YOUR CLEARED PRESENTATION/PUBLICATION TO DTIC NOTE: All publications/presentations are required to be placed in the Defense Technical Information Center (DTIC).			
DATE March 18, 2016			
13. 59 MDW PRIMARY POINT OF CONTACT (Last Name, First Name, M.I., email) Corpus, Raul S raul.s.corpus.ctr@mail.mil			14. DUTY PHONE/PAGER NUMBER 210.539.4404
15. AUTHORSHIP AND CO-AUTHOR(S) List in the order they will appear in the manuscript.			
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f. Davis Michael R	O-5	59MDW ST	
I CERTIFY ANY HUMAN OR ANIMAL RESEARCH RELATED STUDIES WERE APPROVED AND PERFORMED IN STRICT ACCORDANCE WITH 32 CFR 219, AFMAN 40-401 JP, AND 59 MDW 41-100. I HAVE READ THE FINAL VERSION OF THE ATTACHED MATERIAL AND CERTIFY THAT IT IS AN ACCURATE MANUSCRIPT FOR PUBLICATION AND/OR PRESENTATION.			
16. AUTHOR'S PRINTED NAME, RANK, GRADE Sharon Lawson VOL		17. AUTHOR'S SIGNATURE 	18. DATE 15 Mar 2016
19. APPROVING AUTHORITY'S PRINTED NAME, RANK, TITLE Michael R. Davis, O-5, Director RESTOR, Deputy Commander, USA		20. APPROVING AUTHORITY'S SIGNATURE 	21. DATE 16 Mar 2016

PROCESSING OF PROFESSIONAL MEDICAL RESEARCH/TECHNICAL PUBLICATIONS/PRESENTATIONS			
1st ENDORSEMENT (59 MDW/SGVU Use Only)			
10. Clinical Research Division 59 MDWCRD Contact: 292-7141 for email instructions.	22. DATE RECEIVED <div style="text-align: center;">2/16/2016</div>	23. ASSIGNED PROCESSING REQUEST FILE NUMBER <div style="text-align: center;">161-59</div>	
24. DATE REVIEWED	25. DATE FORWARDED TO 502 ISG/JAC		
26. AUTHOR CONTACTED FOR RECOMMENDED OR NECESSARY CHANGES: <input checked="" type="checkbox"/> NO <input type="checkbox"/> YES If yes, give date: <input type="checkbox"/> N/A			
27. COMMENTS <input checked="" type="checkbox"/> APPROVED <input type="checkbox"/> DISAPPROVED <div style="margin-top: 5px;"> <p>- Title Slide lists CPT Cindass as Presenter while Form 3039 does not list him at all. Please correct Form 3039 or change title slide to reflect authorship listed on 3039.</p> </div>			
28. PRINTED NAME, RANK/GRADE, TITLE OF REVIEWER <div style="margin-top: 5px;">Linda D Harris GS-14 Chief, Ops Br</div>	29. REVIEWER SIGNATURE <div style="margin-top: 5px;"><i>Linda D Harris</i></div>	30. DATE <div style="margin-top: 5px;">16 Mar 16</div>	
2nd ENDORSEMENT (502 ISG/JAC Use Only)			
31. DATE RECEIVED	32. DATE FORWARDED TO 59 MDW/WPA		
33. COMMENTS <input checked="" type="checkbox"/> APPROVED (In compliance with security and policy review directives.) <input type="checkbox"/> DISAPPROVED <div style="margin-top: 10px;"> <p>Slide presentation includes disclaimer required by the Joint Ethics Regulation. There are no ethics issues with making this presentation at the 2016 Society of Military Surgeons Meeting.</p> </div>			
34. PRINTED NAME, RANK/GRADE, TITLE OF REVIEWER <div style="margin-top: 5px;">Holly J. Mackey, GS-13</div>	35. REVIEWER SIGNATURE <div style="margin-top: 5px;"><i>Holly J Mackey</i></div>	36. DATE <div style="margin-top: 5px;">16 Mar 2016</div>	
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37. DATE RECEIVED <div style="margin-top: 5px; text-align: center;">17 March 2016</div>	38. DATE FORWARDED TO 59 MDW/SGVU <div style="margin-top: 5px; text-align: center;">18 March 2016</div>		
39. COMMENTS <input checked="" type="checkbox"/> APPROVED (In compliance with security and policy review directives.) <input type="checkbox"/> DISAPPROVED			
40. PRINTED NAME, RANK/GRADE, TITLE OF REVIEWER <div style="margin-top: 5px;">Christopher Carwile, TSgt/E-6, NCOIC, PA</div>	41. REVIEWER SIGNATURE <div style="margin-top: 5px;"> <small>Digitally signed by CARWILE CHRISTOPHER STEWART 1280477229 DN: cn=US, ou=5. Quartermaster, o=DA, ou=USAF, email=CARWILE CHRISTOPHER STEWART 1280477229, Date: 2016.03.18 11:08:21 -0500</small> <i>CARWILE CHRISTOPHER STEWART 1280477229</i> </div>	42. DATE <div style="margin-top: 5px;">18 March 2016</div>	
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43. DATE RECEIVED	44. SENIOR AUTHOR NOTIFIED BY PHONE OF APPROVAL OR DISAPPROVAL <input type="checkbox"/> YES <input type="checkbox"/> NO <input type="checkbox"/> COULD NOT BE REACHED <input type="checkbox"/> LEFT MESSAGE		
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Locally applied enzyme activated tacrolimus eluting hydrogels significantly delay the onset of acute rejection of VCA grafts

** This person should be the Primary Author on Form 3039*
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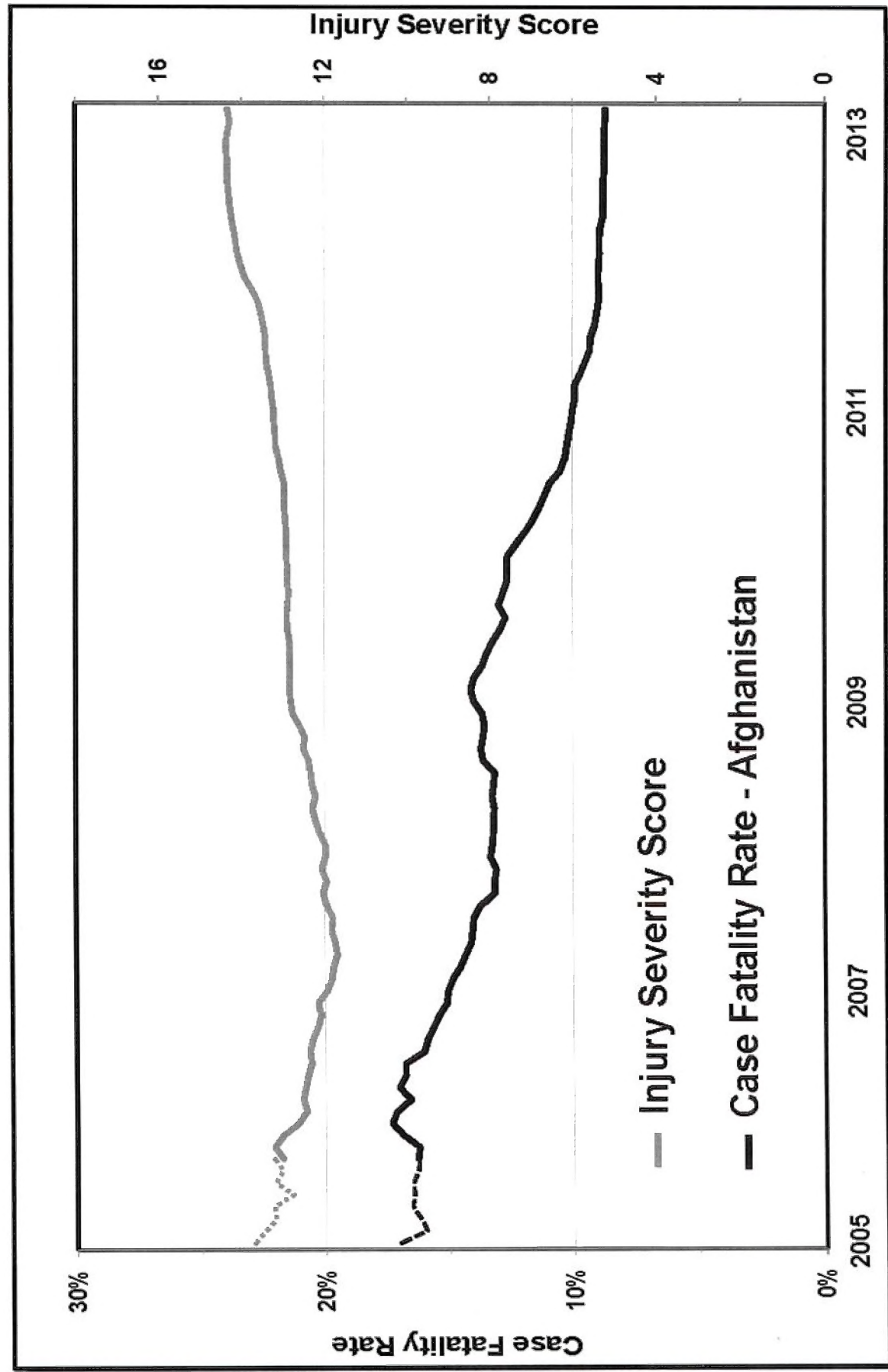
Disclaimer

The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of Defense.

The experiments reported herein were conducted according to the principles set forth in the National Institute of Health Publication No. 80-23, Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966, as amended



Impact of Military Trauma Care and Research







New rung on the reconstructive ladder

Vascularized composite
allotransplantation

Free tissue transfer
eg, latissimus dorsi flap

Regional flaps
eg, posterior interosseous

Local flap
eg, rotational/transposition

Skin graft

Secondary closure

Primary closure



Vascularized Composite Allotransplantation (VCA)

- Multiple types of tissues are transplanted as a single functional unit
- Replaces like with like and restores form and function
- Eliminates autologous donor site morbidity and minimizes the need for multiple reconstructive procedures



Vascularized Composite Allotransplantation (VCA)

- Current challenges and limitations
 - A life-enhancing but not a life-saving procedure
 - Requires lifelong systemic immunosuppression
 - Opportunistic infections: 88%
 - Metabolic complications: 70%
 - ≥ 1 episode of acute rejection within 1st year: 85%
 - Limited to highly motivated patients
 - Limited donor pool



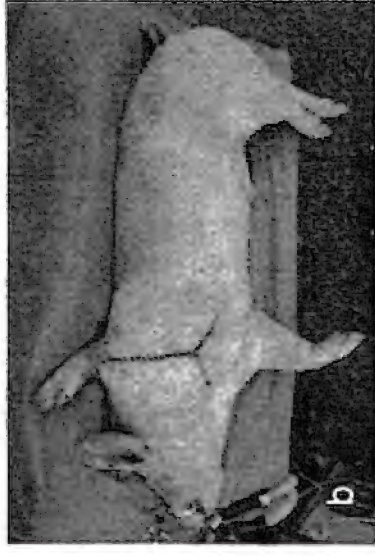
Background

- A novel model of VCA
- Evaluation of a drug eluting hydrogel



A New Model

- Small animal models are technically challenging and lack immunologic maturity
- Previous orthotopic models in non-human primates and canines are no longer in use
- Currently only heterotopic swine models exist



Hettiaratchy, S., Melendy, E., Randolph, M. A., Coburn, R. C., Neville, D. M., Sachs, D. H., et al. (2004). Tolerance to composite tissue allografts across a major histocompatibility barrier in miniature swine. *Transplantation*, 77(4), 514–521.

Leto Barone, A. A., Leonard, D. A., Torabi, R., Mallard, C., Glor, T., Scalea, J. R., et al. (2013). The gracilis myocutaneous free flap in swine: an advantageous preclinical model for vascularized composite allograft transplantation research. *Microsurgery*, 33(1), 51–55

Kiermeir, D. M., Meoli, M., Müller, S., Abderhalden, S., Vögelin, E., & Constantinescu, M. A. (2013). Evaluation of a porcine whole-limb heterotopic autotransplantation model. *Microsurgery*, 33(2), 141–147

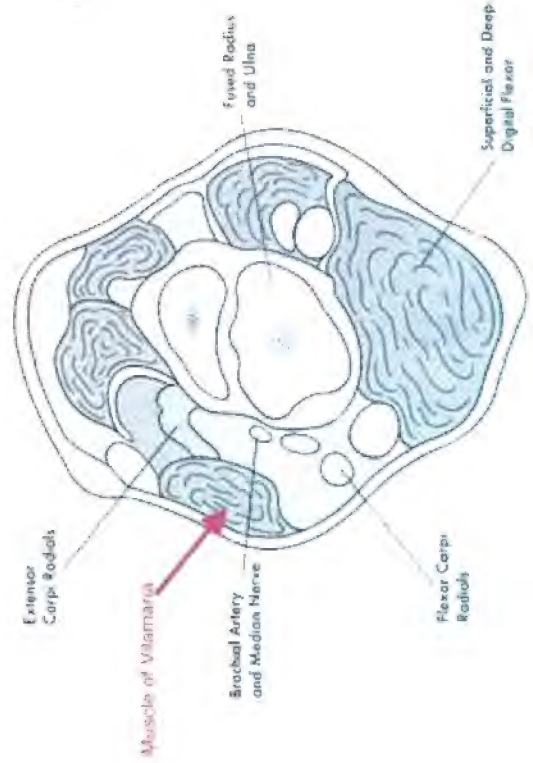
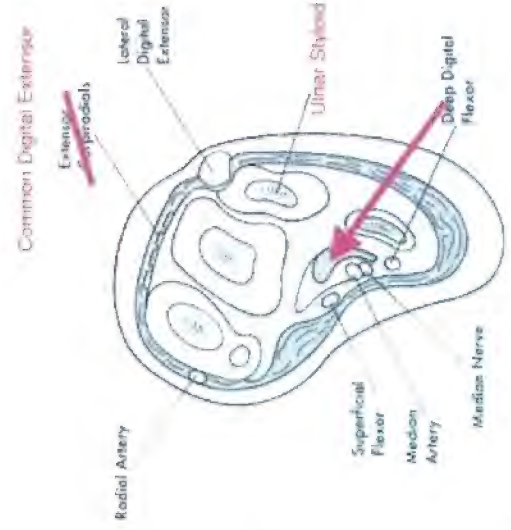
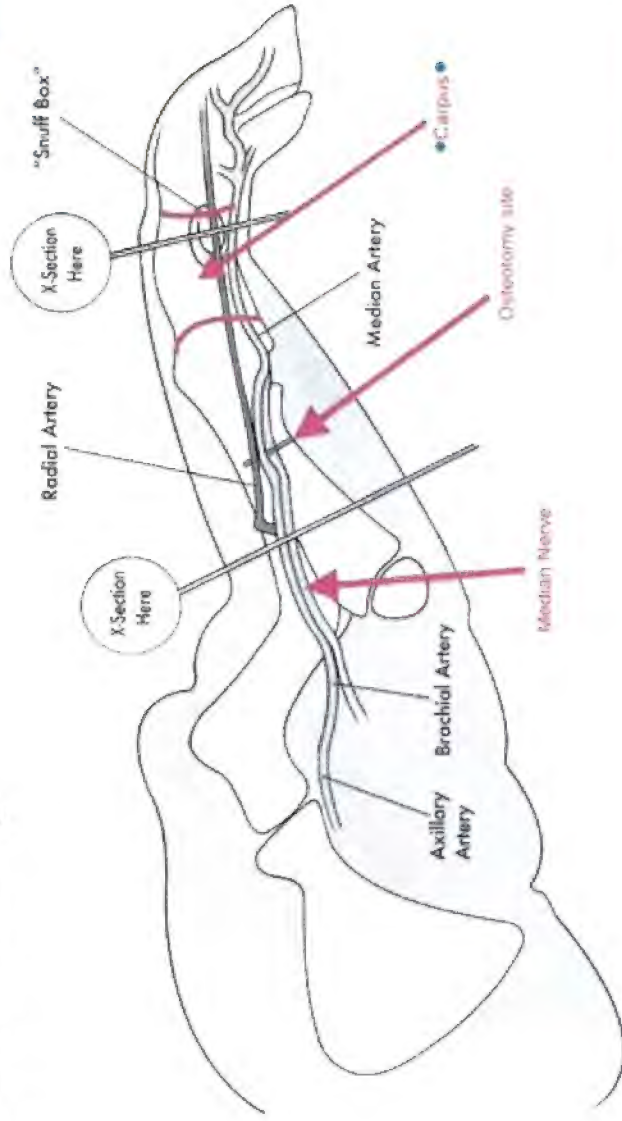


A New Model

- Ethically acceptable
- Reproducible
- Genetically controlled animals
- Orthotopic model to assess functionality
- Evaluation of bone, tendon and nerve healing

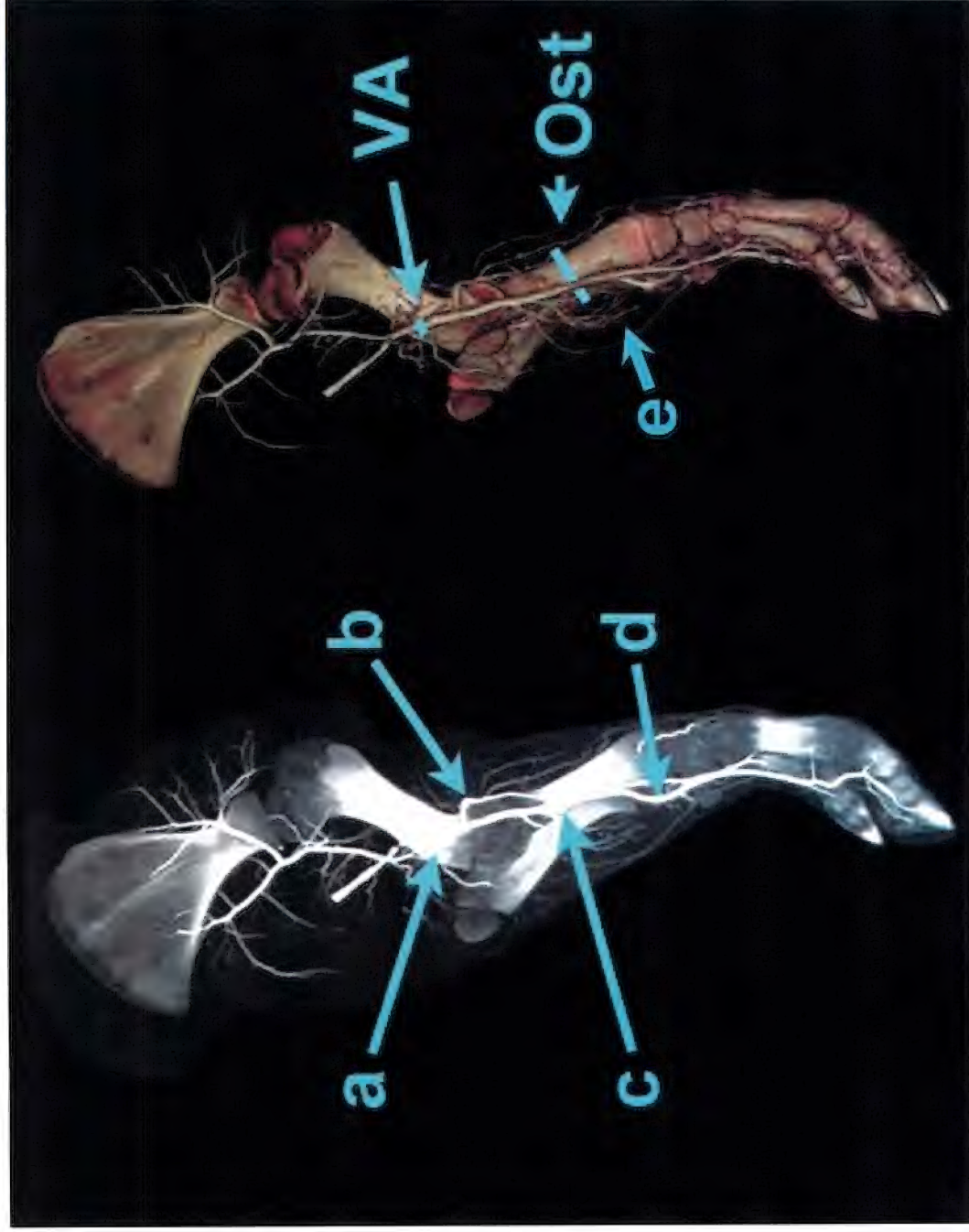


Anatomy





Anatomy



- A – Axillary Artery
- B – Radial Artery
- C – Interosseous Branch (of Fries)
- D – Median Artery
- E – The Nest (of Lawson)

- VA – Vascular Anastomosis
- Ost – Osteotomy Site



2 Weeks Post-op





Enzyme Activated Drug Eluting Hydrogel

RESEARCH ARTICLE

TRANSPLANTATION

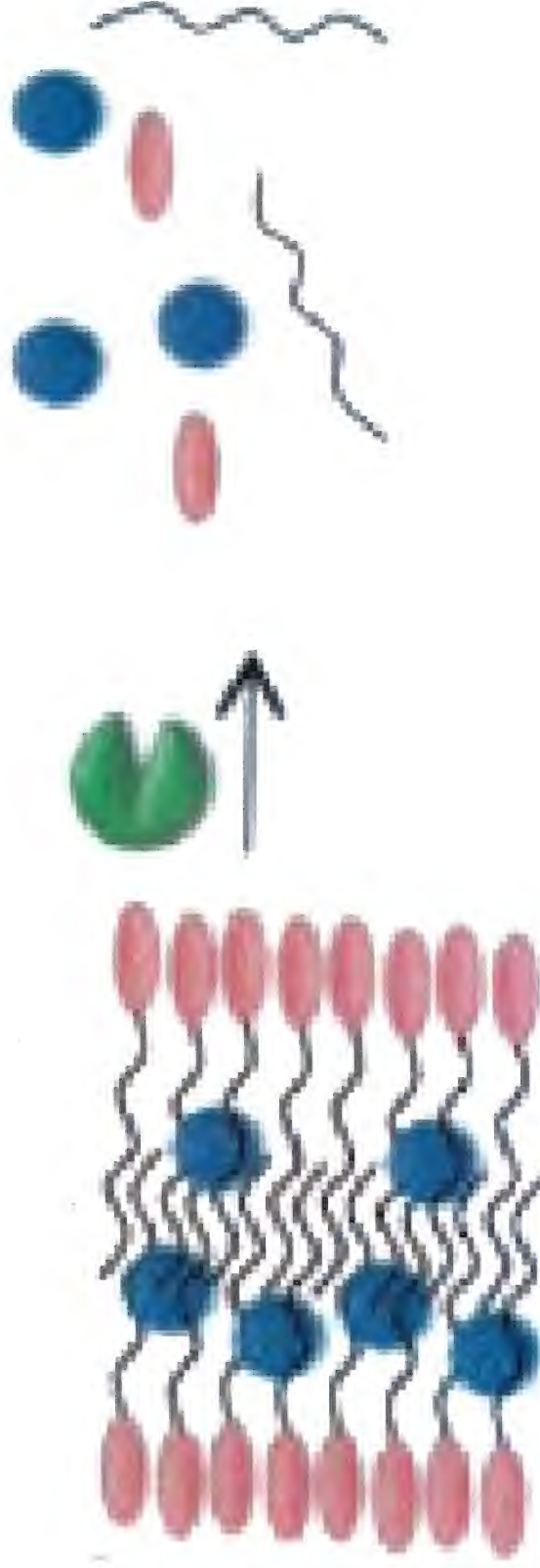
A single localized dose of enzyme-responsive hydrogel improves long-term survival of a vascularized composite allograft

Thusitha Gajanayake,^{1,2*} Radu Olariu,^{1,2*} Franck M. Leclère,^{1,2} Ashish Dhayani,³ Zijiang Yang,⁴ Anjan K. Bongoni,^{2,5} Yara Banz,⁶ Mihai A. Constantinescu,^{1,2} Jeffrey M. Karp,^{4†} Praveen Kumar Vemula,^{3†} Robert Rieben,^{2†} Esther Vögelin^{1,2}

Currently, systemic immunosuppression is used in vascularized composite allotransplantation (VCA). This treatment has considerable side effects and reduces the quality of life of VCA recipients. We loaded the immunosuppressive drug tacrolimus into a self-assembled hydrogel, which releases the drug in response to proteolytic enzymes that are overexpressed during inflammation. A one-time local injection of the tacrolimus-laden hydrogel significantly prolonged graft survival in a Brown Norway-to-Lewis rat hindlimb transplantation model, leading to a median graft survival of >100 days compared to 33.5 days in tacrolimus only-treated recipients. Control groups with no treatment or hydrogel only showed a graft survival of 11 days. Histopathological evaluation, including anti-graft antibodies and complement C3, revealed significantly reduced immune responses in the tacrolimus-hydrogel group compared with tacrolimus only. In conclusion, a single-dose local injection of an enzyme-responsive tacrolimus-hydrogel is capable of preventing VCA rejection for >100 days in a rat model and may offer a new approach for immunosuppression in VCA.



Enzyme Activated Drug Eluting Hydrogel





Methods

- Three groups
 - Group 1: Controls – no immunosuppression
 - Group 2: High dose tacrolimus eluting hydrogel (84mg)
 - Group 3: Low dose tacrolimus eluting hydrogel (49mg)
- 1 swine leukocyte antigen (SLA) donor-recipient mismatch
- No systemic immunosuppression
- Hydrogel injected in the subcutaneous layer following revascularization
- AST, LDH, CK, TNF-a, IL-6, myoglobin, and biopsies were assessed for signs of systemic toxicity and/or acute rejection
- End-point – Banff grade 4 acute rejection or 100 days



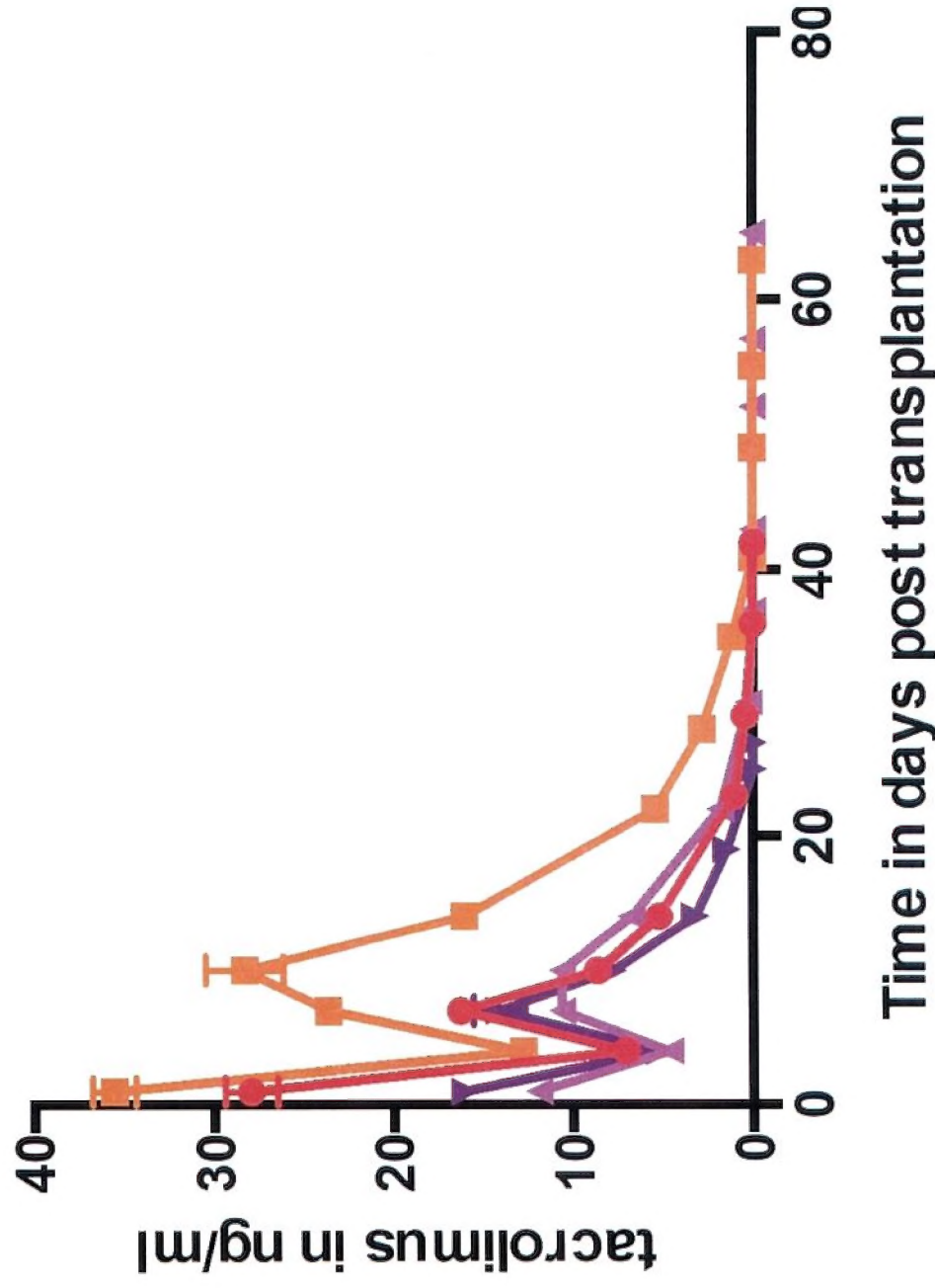
The graph displays the progression of rejection grades over 80 post-operative days. The Control group (blue) remains at grade 1. High dose Tac groups (purple) show a general downward trend from grade 3 to 0. Low dose Tac groups (red) show more variability, with some reaching grade 0 and others remaining at grade 1 or 2. POD2 Death is indicated by a black line with a cross at day 0. Failure of revascularization is indicated by a black line with a diamond at day 0.

Time / post op days	Control (Blue)	High dose Tac (Purple)	Low dose Tac (Red)	POD2 Death	Failure of revascularization
0	1	0	0	0	0
10	1	0	0	0	0
20	1	0	0	0	0
30	1	0	0	0	0
40	1	0	0	0	0
50	1	0	0	0	0
60	1	0	0	0	0
70	1	0	0	0	0
80	1	0	0	0	0



Results

Tacrolimus systemic levels





Conclusions

- The orthotopic model of swine VCA is an optimal model for investigating novel immunologic strategies
- Hydrogels are able to delay the onset of acute rejection with no gross safety concerns and without clinically detectable systemic levels of tacrolimus



Future direction

- Further hydrogel protocols to establish optimal dosing regimen and potential protocols for re-loading hydrogels
- Increased survival duration to evaluate longer term rejection and side effects profile



Thank you

USAISR/59MDW

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Lt Col Dmitry Tudor
Dr Shari Lawson
Dr Kevin Wu
CPT Lin Wang
Mr Raul Corpus

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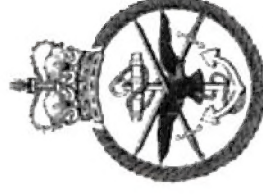
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